HMGB1 PROTEIN DERIVATIVES FOR THE REMOVAL OF BIOFILMS

~~CROSS-REFERENCE TO RELATED APPLICATION~~

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[0001] This application claims priority under 35 U.S.C. § 119(e) to U.S. Provisional Application Serial No.: 62/742,102, filed October 5, 2018, the contents of which are hereby incorporated by reference in its entirety.

STATEMENT OF FEDERAL SUPPORT

[0002] This invention was made with government support under Grant No. DC011818 awarded by the National Institutes of Health (NIH). The government has certain rights in the invention.

FIELD OF THE DISCLOSURE

[0003] This invention generally relates to the methods and compositions to lessen and/or cure clinical or industrial bacterial biofilms.

BACKGROUND

[0004] Bacteria persisting in a biofilm in the human body cause about two-thirds of all chronic/recurrent diseases. These biofilms are comprised of bacteria protected by an outer "slime" that is often comprised primarily of DNA which prevents the innate and adaptive immune systems, antibiotics and other antibacterial agents from gaining access to the bacteria inside the biofilm. Biofilms make it extremely difficult to clear the infection from the body. Furthermore, biofilms can act as a reservoir for future acute infections often with lethal consequences.

[0005] At least one protein from the DNABII family of proteins is found in all known eubacteria and are naturally found outside of the bacterial cell. While they elicit a strong innate immune response, host subjects fail to naturally produce specific antibody to family members as a result of infection. The major problem with bacterial biofilms is the inability of the host immune system and/or antibiotics and other antimicrobials to gain access to the bacteria protected within the biofilm.

[0006] Biofilms are present in an industrial setting as well. For example, biofilms are implicated in a wide range of petroleum process problems, from the production field to the gas station storage tank. In the field, sulfate reducing biofilm bacteria produce hydrogen sulfide (soured oil). In the process pipelines, biofilm activity develops slimes which impede

filters and orifices. Biofilm and biofilm organisms also cause corrosion of pipeline and petroleum process equipment. These problems can be manifested throughout an oil or gas production facility to the point where fouling and corrosive biofilm organisms have even been found on the surfaces of final product storage tanks.

[0007] In the home, biofilms are found in or on any surface that supports microbial growth, e.g., in drains, on food preparation surfaces, in toilets and in swimming pools and spas.

[0008] Biofilms are implicated in a wide range of water processes, both domestic and industrial. They can grow on the surface of process equipment and impede the performance of the equipment, such as degradation of heat transfer or plugging of filters and membranes. Biofilms growing on cooling tower fill can add enough weight to cause collapse of the fill. Biofilms cause corrosion of even highly specialized stainless steels. Biofilms in a water process can degrade the value of a final product. Biofilms growing in drinking water distribution systems can harbor potential pathogenic organisms, corrosive organisms or bacteria that degrade the aesthetic quality of the water.

[0009] Thus, a need exists to break through the protective barrier of biofilms to treat or kill the associated bacterial infections and clear them from surfaces and in water systems. This disclosure satisfies this need and provides related advantages as well.

SUMMARY

[009a] In one aspect, the present disclosure refers to an isolated B Box polypeptide comprising an amino acid mutation at C106 or K114, or an equivalent thereof comprising the amino acid mutation at C106 or K114.

[009b] In another aspect, the present disclosure refers to an isolated A Box polypeptide, or an equivalent thereof comprising an amino acid mutation at an amino acid position selected from K12, C23 or C45.

[009c] In another aspect, the present disclosure refers to an isolated AB Box polypeptide, or an equivalent thereof.

[009d] In another aspect, the present disclosure refers to an antibody that recognizes and binds an isolated polypeptide as described herein or an antigen binding fragment of the antibody.

[009e] In another aspect, the present disclosure refers to a composition comprising a carrier and one or more isolated polypeptide as described herein, or an antibody or antigen binding

fragment that recognizes and binds the isolated polypeptide, or an isolated host cell comprising one of more of: the isolated polypeptide, the antibody or antigen binding fragment, a polynucleotide encoding the isolated polypeptide or the antibody or antigen binding fragment, a complement of the polynucleotide, or a vector comprising the polynucleotide.

[009f] In another aspect, the present disclosure refers to a polynucleotide encoding the isolated polypeptide as described herein or the complement of the polynucleotide.

[009g] In another aspect, the present disclosure refers to a vector comprising the polynucleotide as described herein.

[009h] In yet another aspect, the present disclosure refers to an isolated host cell comprising one of more of: the polypeptide as described herein, an antibody or antigen binding fragment that recognizes and binds the isolated polypeptide, a polynucleotide encoding the isolated polypeptide or the antibody or antigen binding fragment, a complement of the polynucleotide, or a vector comprising the polynucleotide.

[009i] In yet another aspect, the present disclosure refers to a method for inhibiting, competing or titrating the binding of a DNABII polypeptide or protein to a microbial DNA, comprising contacting the DNABII polypeptide or protein or the microbial DNA with the polypeptide as described herein, thereby inhibiting, competing or titrating the binding of the DNABII protein or polypeptide to the microbial DNA.

[009j] In yet another aspect, the present disclosure refers to a method for inhibiting, preventing or breaking down a microbial biofilm, comprising contacting the biofilm with the polypeptide as described herein, thereby inhibiting, preventing or breaking down the microbial biofilm.

[009k] In yet another aspect, the present disclosure refers to a use of the polypeptide as described herein in the manufacture of a medicament for one or more of the following: (a) inhibiting, preventing or breaking down a biofilm in a subject, (b) inhibiting, preventing or treating a microbial infection that produces a biofilm in a subject, (c) disrupting a biofilm and clearance thereof that does not enhance or induce an inflammatory response in a subject in need thereof, or (d) treating an infection or disorder incident to the presence of a biofilm in a subject in need thereof,

wherein the polypeptide is to be administered into the subject, thereby (a') inhibiting, preventing or breaking down the microbial biofilm, (b') inhibiting, preventing or treating a

microbial infection that produces the biofilm in the subject, (c') disrupting the biofilm and clearance thereof that does not enhance or induce an inflammatory response, or (d') treating the infection or disorder incident to the presence of a biofilm in the subject, respectively.

[009l] In yet another aspect, the present disclosure refers to a kit comprising one or more of: the isolated polypeptide as described herein, an antibody or antigen binding fragment that recognizes and binds the isolated polypeptide, a polynucleotide encoding the isolated polypeptide or the antibody or antigen binding fragment, or a complement of the polynucleotide and instructions for use.

[009m] In yet another aspect, the present disclosure refers to the polypeptide as described herein for use as a therapy.

[0010] Bacterial biofilms are notoriously recalcitrant to existing treatment modalities (for example they are >1000 fold more resistant to antimicrobials than their planktonic counterparts). Given the high prevalence and the enormous consequences in terms of attributable mortality and economic burden of biofilm-mediated infections, novel therapeutic approaches are urgently needed. One of the defining characteristics of a biofilm is the extracellular polymeric substance, in which biofilm cells are embedded. Key components of the extracellular polymeric substance are extracellular DNA and bacterial DNABII family of proteins, which are crucial to biofilms' structural integrity. Targeting and sequestration of DNABII proteins can disrupt biofilms. High Mobility Group B1 (HMGB1) protein is a DNA-binding eukaryotic protein that binds to the same DNA structures as the DNABII proteins, causing, disruption of bacterial biofilms. Derivatives of HMGB1 can be engineered to possess the same efficacious anti-biofilm activity but are smaller and do not induce inflammation.

[0011] Applicant discloses herein a new concept in the treatment of bacterial biofilm-mediated infections, by repurposing derivatives of an innate immune effector. HMGB1 domains are functionally different. Domain variants with anti-biofilm activities and no pro-inflammatory outcomes represent one ~~embodiment~~example of HMGB1 to treat biofilm-mediated infections without the consequences of excessive inflammation. *In vivo* and *ex vivo* experiments showed that antibodies against the DNABII family of bacterial nucleoid-associated proteins (IHF and HU) are highly effective against many different bacterial biofilms that cause a variety of recalcitrant human infections. In contrast, this disclosure utilizes HMGB1, an immune response component, to treat biofilm-mediated diseases without the consequence of excessive inflammation. *In vitro* bacterial biofilms were exposed to the anti-biofilm properties of HMGB1 and its various truncated domains (A box, B box, B box linker, mutated B box C106S, AB boxes and all with linkers).

[0012] The compositions and formulations containng the protein derivatives are useful in the treatment of recalcitrant or chronic or recurrent biofilm-mediated infections. The compositions are useful to treat resistant nosocomial infections (including indwelling medical device-related infections such as catheter- or prosthetic device-related infections, and chronic/recurrent infections such as ear infections and respiratory tract infections in cystic fibrosis patients). Additionally, they can be used in combination with established treatments (i.e antibiotics) as it has been shown that many bacteria released from biofilms are more susceptible to both host defenses and antimicrobial agents.

[0013] Thus, in one ~~aspect~~example, this disclosure provides an isolated A Box polypeptide, optionally comprising, or alternatively consisting essentially of, or yet consisting of, one or more amino acid mutations selected from K12, C23 and C45 (e.g. the native K or C modified to an amino acid from the group selected from serine, glycine, alanine, valine, isoleucine or threonine) or an equivalent thereof, the equivalent comprising one or more amino acid mutations selected from K12, C23 and C45 e.g. the native K or C modified to an amino acid from the group selected from serine, glycine, alanine, valine, isoleucine or threonine. In one ~~aspect~~example, the mutation is a C45S mutation. The A Box polypeptide may futher comprise a linker or peptide sequence located at one or both termini. A non-limiting example is a polypeptide linker of the sequence PPKGETKKKF. When recombinantly produced, theB Box polypeptides can be partially or fully acetylated, oxidized or phosphorylated. In one ~~aspect~~example, the A Box polypeptide comprises, or consists essentially of, or yet further consists of

amino acids 1 to 70 of wild-type HMGB1 polypeptide that optionally contains one or more mutations as identified above.

[0014] Also provided herein is an isolated B Box polyeptide, optionally comprising, or alternatively consisting essentially of, or yet consisting of a mutation at amino acid C106 (e.g. the native cysteine to an amino acid from the group selected from serine, glycine, alanine, valine, isoleucine or threonine), or an equivalent thereof comprising a mutation at amino acid C106 (e.g. the native cysteine to an amino acid from the group selected from serine, glycine, alanine, valine, isoleucine or threonine). In one ~~aspect~~example, the B Box polypeptide comprises, or consists essentially of, or yet further consists of amino acids about 80 to about 176, or about 88 to about 164, or about 89 to about 162, or yet further about 80 to about 164, of the HMGB1 polypeptide. Additional locations for modification of the wild-type HMGB1 B Box polypeptide are shown in **FIG. 1C**.

[0015] The B Box polypeptide may futher comprise a linker or peptide sequence located at one or both termini. A non-limiting example is a polypeptide linker of the sequence PPKGETKKKF. When recombinantly produced, the disclosed B Box polypeptides can be partially or fully acetylated, oxidized or phosphorylated.

[0016] In a further ~~aspect~~example, provided herein is an isolated AB Box polypeptide, optionally comprising, or alternatively consisting essentially of, or yet consisting of, one or more amino acid mutations selected from K12, C23, C45, or C106 (e.g. the native K or C modified to an amino acid from the group selected from serine, glycine, alanine, valine, isoleucine or threonine) or an equivalent thereof comprising one or more amino acid mutations selected from K12, C23 and C45 (e.g. the native K or C modified to an amino acid from the group selected from serine, glycine, alanine, valine, isoleucine or threonine). In one ~~aspect~~example, the mutation is a C45S mutation. In another ~~aspect~~example, the polypeptide comprises a mutation at amino acid C106 (e.g. the native cysteine to an amino acid from the group selected from serine, glycine, alanine, valine, isoleucine or threonine), or an equivalent thereof comprising one or more amino acid mutations selected from K12, C23, C45 and a mutation at amino acid C106 (e.g. the native cysteine to an amino acid from the group selected from serine, glycine, alanine, valine, isoleucine or threonine). In one ~~aspect~~example, the AB Box polypeptide and equivalents comprise C45S and C106S mutations. In one ~~aspect~~example, the AB Box polypeptide or its equivalent comprises, or consists essentially of, or yet further consists of amino acids 1 to

176, or 1 to 162, or yet further 1 to 164, of the wild type HMGB1 polypeptide, with the noted amino acid mutations.

[0017] In a yet further ~~aspect~~example, the isolated AB Box polypeptide of further comprises a linker polypeptide located linking the A Box polypeptide and the B Box polypeptide and in one ~~aspect~~example, a second linker linking the B Box and a C Box polypeptide. A non-limiting example is a polypeptide linker of the sequence PPKGETKKKF. When recombinantly produced, the AB or A, B and C Box polypeptides can be partially or fully acetylated, oxidized or phosphorylated. In one ~~aspect~~example, an isolated mutated HMGB1 polypeptide is provided with 1 or more amino acid subsitutions as described herein, in the A and/or B box domains that can optionally be partially or fully acetylated, oxidized or phosphorylated.

[0018] In one ~~aspect~~example, the isolated polypeptides further comprise a detectable label.

[0019] Also provided herein is a recombinant polypeptide comprising, or alternatively consisting essentially of, or yet consisting of, one or more of the isolated polypeptides as described herein, further comprising at least one additional amino acid located at either or both termini.

[0020] This disclosure also provides an antibody that binds to, or was raised against a mutated polypeptide as described herein. The antibodies are useful as diagnostic and prognostic agents. Further provided one or more isolated polypeptides and/or antibodies as described herein and a carrier, such as a pharmaceutically acceptable carrier.

[0021] This disclosure also provides polynucleotides encoding the isolated polypeptide or antibody as described herein as well as their complements. In one ~~aspect~~example, the polynucleotides are detectably labeled. The polynucleotides can optionally be operatively linked to a promoter and/or enhancer for expression of the polynucleotide. Further provided is a method of recombinantly producing the polypeptides by expressing the polynucleotides in an appropriate expression system such as a host cell, and then producing and isolating the recombinantly produced polypeptides.

[0022] Yet further provided is a vector comprising, or alternatively consisting essentially of, or yet consisting of, a polynucleotide as described herein.

[0023] In another ~~aspect~~example, provided herein is an isolated host cell comprising one of more of a polypeptide, a polynucleotide, or a vector as described herein. Compositions comprising a

carrier and one of more of a polypeptide, a polynucleotide, or a vector as described herein are further provided. In one ~~aspect~~example, the carrier is a pharmaceutically acceptable carrier.

[0024] The polypeptides and compositions comprising them have multiple uses. For example, the can be used in a method for inhibiting, competing or titrating the binding of a DNABII polypeptide or protein to a microbial DNA by contacting the DNABII polypeptide or protein or the microbial DNA with the polypeptide or composition as described herein. They also can be used in methods for inhibiting, preventing or breaking down a microbial biofilm by contacting the biofilm with the polypeptide or composition as described herein.

[0025] The polypeptide and compostions also can be used in methods of inhibiting, preventing or breaking down a biofilm in a subject or treating an infection or disease associated with the biofilm, by administering to the subject an effective amount of the composition or polypeptide as described herein.

[0026] The polypeptides and compositions can further be used in methods for inhibiting, preventing or treating a microbial infection that produces a biofilm in a subject, by administering to the subject an effective amount of the composition or polypeptide as described herein.

[0027] The methods can further comprise contacting or administering an effective amount of an an additional agent, such as an antimicrobial agent to treat the underlying infection.

[0028] The biofilms and infections that can be treated by these methods can be caused by bacterial infections, e.g., infections by ESKAPE pathogens, uropathogenic *Escherichia coli* (UPEC), *Klebsiella pneumonia*, *Burkholderia cenocepacia, S. epidermidis*, *Streptococcus agalactiae, Neisseria meningitidis, Treponemes, denticola, pallidum), Burkholderia cepacia*, *Burkholderia pseudomallei*, *Haemophilus influenzae* (nontypeable)(NTHI), *Moraxella catarrhalis*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, or *Mycobacterium tuberculosis.* Device related infections caused by biofilms include, for example, ventricular derivations, on contact lens, on endotracheal tubes, on prosthetic cardiac valves, pacemakers, and vascular grafts, on tissue fillers and breast implants, on peripheral vascular catheters, on urinary cathetes, on orthopedic implants and prosthetic joints. Tissue-related infections that can be treated by the compositions and methods include for example, chronic otitis media, chronic sinusitis, chronic tonsillitis, dental plaque, chronic laryngitis, endocarditis, lung infections (infections upper, mid and lower airway (otitis, sinusitis, bronchitis but also exacerbations of chronic obstructive pulmonary disease (COPD), chronic